Table 10 Effect of collector type on Cu-Pb-Zn-Au metallurgical results from a high lead ore

Collector used	*Product*	*Weight (%)*	*Assays (%, g t^{-1})*				*Distribution (%)*			
			Au	*Cu*	*Pb*	*Zn*	*Au*	*Cu*	*Pb*	*Zn*
Xanthate = 30 g/t	Cu concentrate	2.47	22.4	25.5	1.20	4.50	41.6	78.6	2.3	1.3
Dithiophosphate	Pb concentrate	1.80	2.50	0.80	51.5	8.30	3.4	1.8	71.3	1.7
3477 = 20 g/t	Zn concentrate	13.94	1.10	0.60	0.80	58.2	11.5	10.4	8.6	92.2
	Tailing	81.79	0.71	0.089	0.28	0.52	43.5	9.2	17.8	4.8
	Feed	100.00	1.33	0.80	1.30	8.80	100.0	100.0	100.0	100.0
Xanthate = 30 g/t	Cu concentrate	2.52	31.3	26.1	1.10	5.00	60.6	80.1	2.1	1.4
Aerophine 3418A = 20 g/t	Pb concentrate	1.92	2.80	0.90	51.1	9.20	4.1	2.1	72.5	2.0
	Zn concentrate	13.91	0.90	0.50	0.72	58.5	9.6	8.5	7.4	92.5
	Tailing	81.65	0.41	0.093	0.30	0.44	25.7	9.3	18.0	4.1
	Feed	100.00	1.30	0.82	1.35	8.80	100.0	100.0	100.0	100.0

mineralogy, gangue composition and gold particle size. There is no universal method for flotation of the gold-bearing minerals, and the process is tailored to the ore characteristics. A specific reagent scheme and flow sheet are required for each ore.

- There are opportunities on most operating plants for improving gold metallurgy. Most of these improvements come from selection of more effective reagent schemes, including collectors and modifiers.
- Perhaps the most difficult ores to treat are the clay-containing carbonaceous sulfides. Significant progress has been made in treatment options for these ores. New sulfide activators (e.g. amine-treated $CuSO_4$, ammonium salts) and nitrogen gas flotation are amongst the new methods available.

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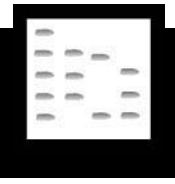
GRADIENT POLYMER CHROMATOGRAPHY: LIQUID CHROMATOGRAPHY



G. Glöckner, Dresden University of Technology, Dresden, Germany

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Classical Precipitation Chromatography

Polymer Solubility and Precipitation

Solubility is governed by the general requirement that the change in Gibbs' free energy must be negative. With low molecular weight substances this condition is easily fulfilled, because the entropy contribution is large owing to the large number of particles involved. But with polymer compounds, the entropy of dissolution is comparatively small and the enthalpy contribution gains in importance. The precept that '*similia similibus solventur*' becomes a stringent requirement; in terms of Hildebrand's solubility concept, this means that a polymer can dissolve only in fluids whose solubility parameters are very closely related

to those of the polymer. Therefore, most liquids are non-solvents and the number of solvents available for a given polymer is far fewer than the number of solvents available for a low molecular weight substance of comparable structure.

The solubility of polymers decreases with increasing molecular weight (MW) and can be measured easily by the controlled addition of a non-solvent to the solution of a polymer. The volume fraction ϕ_{NS} of non-solvent at the cloud point is related to the square root, $M^{0.5}$, of molecular weight by

$$100\ \phi_{NS} = C_1 + C_2/M^{0.5}$$

where C_1 and C_2 are constants for the particular system.

This dependence can be used to separate polymers by either fractional precipitation or dissolution. The latter method can also be performed in packed columns by gradients whose solvent power increases in the course of the elution.

The solubility of polymers also depends on temperature. Usually, the temperature coefficient is positive, i.e. fractional dissolution can be carried out with a given solvent (or a non-solvent/solvent mixture at constant composition) by raising the temperature. This procedure can also be performed in columns.

Baker–Williams Fractionation

In 1956, Baker and Williams described 'a new chromatographic procedure and its application to high polymers'. This was column elution combining the effects of solvent strength and temperature. The important innovation was a temperature gradient along the column. The top of the column was heated to a temperature about 50 K higher than that of the cooled bottom. An aluminium jacket ensured a linear temperature profile. The polymer to be investigated was coated onto the part of the inert packing that subsequently was put into the uniformly heated uppermost section of the column. The temperature gradient enabled multistage separation to be performed. Any component dissolved from the sample bed was reprecipitated in a cooler zone of the column. Here it was redissolved later by a non-solvent/solvent mixture of higher solvent strength and transported to the next cooler zone for another reprecipitation. Thus, Baker–Williams fractionation was described as 'a chromatographic method based upon the equilibration of substances between a stationary precipitated phase and a moving solution'. Baker and Williams investigated polystyrene in a glass tube, 350 mm long and 24 mm wide, packed with glass beads of average size 0.1 mm diameter. The sample size was 300 mg, the gradient ran from ethanol (non-solvent) to methyl ethyl ketone, and the temperature gradient spanned 10–60°C. The multistage mechanism ensured a high separation power, which was confirmed by both theory and experiment. The method became popular in polymer characterization; the second citation in the Bibliography provides a survey of application of the method to about 30 different polymers.

The development of size exclusion chromatography (SEC) made separation according to MW feasible and convenient. SEC allows the investigation of different polymers in a common eluent with very little preliminary work. Dissolved samples can be injected into a running eluent, e.g. tetrahydrofuran, which has sufficient solvent strength for a great many polymers. The elution curve can be monitored with a suitable detector and provides at least a first guess at the MW distribution (MWD). Using MW-sensitive detectors and sophisticated software, reliable MWD curves can be measured within minutes. Interest in the demanding Baker–Williams technique therefore faded away. Although this technique is no longer a competitor in analytical separations according to MW, it should still be considered a powerful tool for separations according to chemical differences and for preparative fractionation. The chemical composition distribution of copolymers, blends or modified polymers can be measured by SEC only in rare cases (if coupled with MWD in a known ratio). This was realized some years ago (see Bibliography).

High Performance Precipitation Liquid Chromatography (HPPLC)

Principle and Instrumentation

The renaissance of precipitation chromatography requires modern equipment, e.g. detectors and programmable gradient devices. The samples to be investigated should be applied in solution and injected into the eluent stream ahead of the column.

About 80% of all high performance liquid chromatography (HPLC) investigations are performed in the reversed-phase mode. Reversed-phase packing materials have a nonpolar surface. They usually consist of particles with a silica core and a bonded layer of alkane chains. Reversed-phase gradients run from a highly polar initial eluent to a final eluent of low polarity. The polar eluent forces nonpolar solutes to be retained by the stationary phase. Retention increases with decreasing polarity of the sample components. The mechanism is understood to be a solvophobic interaction that requires the

mobile phase to be an unfavourable environment for the solute.

The measures taken to force the polymer towards the stationary phase may easily reach or even transgress the limits of solubility. The latter effect has been observed occasionally in reversed-phase chromatography of low molecular weight compounds, but is almost the rule with polymers whose solubility is more restricted.

In normal-phase chromatography, the column is polar and gradient elution is performed with a nonpolar starting component A and a polar component B is added during the run. Retention increases with increasing polarity of the sample constituents.

In order to achieve proper retention of a polymer, the starting eluent A must usually be a non-solvent. This means that sample solutions cannot be prepared in a portion of the starting eluent and that the polymer is precipitated at the top of the column. Since proper retention is required, the separation is by this step classified as precipitation chromatography. The precipitation at the top of the column yields preconcentration of the sample. Thus, HPPLC can cope with samples differing widely in concentration, e.g. SEC fractions. The column permeability is not affected. If the sample solvent is a portion of eluent B, the amount of solvent injected will not cause difficulties. The use of another solvent is not recommended because it could overload the column with an additional substance.

The mechanism of separation is, in general, a combination of precipitation and adsorption. The detector must be capable of measuring the eluting sample components without being affected by the solvent gradient. Suitable equipment became available in the late 1970s.

High Performance Precipitation Liquid Chromatography of Styrene–Acrylonitrile Copolymers

Styrene is a polymerizable substance of formula $CH_2{=}CH(C_6H_5)$, whose homopolymerization yields polystyrene (PS). It can be polymerized with numerous other monomers to yield copolymers. Styrene units have a strong UV absorption, which means that polystyrene and styrene-containing copolymers can be monitored by UV detectors. Copolymers of styrene and acrylonitrile are of commercial interest. Well-characterized samples graded in composition are available together with a considerable knowledge of styrene–acrylonitrile dissolution/precipitation behaviour. The polarity of acrylonitrile is higher than that of styrene units. Therefore a separation of styrene–acrylonitrile copolymers according to composition is also a separation into constituents differing in polarity, which is of basic interest in the framework of chromatography. Styrene–acrylonitrile copolymers therefore seemed to be well suited to early studies of high performance precipitation chromatography.

Preliminary studies published in 1982 showed that tetrahydrofuran (THF) has the capacity to separate a mixed styrene–acrylonitrile sample into its constituents, provided that the starting gradient component A enables proper retention of the injected samples. This was achieved by using at least 80% *n*-hexane in THF, i.e. with a non-solvent. The injected polymer was therefore precipitated at the top of the column. The elution characteristic (percentage THF in the eluent versus acrylonitrile content of the sample) was similar to the solubility borderline determined by turbidimetric titration. It was found that equivalent separations could be achieved on a silica column as well as on a nonpolar C_8 column. This surprising result was confirmed in systematic studies performed by Glöckner and van den Berg in 1987 using other polar and nonpolar columns including silica CN bonded phase, small-pore C_{18}, wide-pore C_{18} and μ-Bondagel E1000-10. Thus, the surface of the packings did not actively participate in the separation. It was found that the peak shapes obtained could not be improved further by a temperature gradient along the column (which had been so essential in Baker–Williams fractionation).

Multistage separation without the use of a temperature gradient or interaction with the surface can be achieved on porous packings where the polymer solute is excluded from the pores. The polymer solute then has a higher linear velocity than the eluent, which fills the interstitial volume as well as the pore volume of the column. The polymer bypasses the pores and thus overtakes the eluent which has sufficient solvent strength. The polymer is precipitated and retained until a more powerful eluent reaches its position.

In chromatographic terms, the gradient hexane → THF is a normal-phase gradient, i.e. increasing in polarity. In combination with a polar column, e.g. silica or a CN bonded phase, it forms a standard normal-phase system, which should elute more polar sample constituents after less-polar ones. Thus, the observed efficiency of irregular combinations with nonpolar C_8 or C_{18} columns shows that the separation was not governed by the common polarity rules of chromatography. The separation of styrene–acrylonitrile copolymers was, under the conditions of these studies, dominated by a precipitation mechanism. Another example of precipitation mechanism in styrene–acrylonitrile gradient chromatography is given in the next section. However, it should be

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firmly stated that styrene–acrylonitrile is an exception rather than the rule. In general, gradient chromatography of synthetic polymers is governed by the *combination of precipitation and adsorption*. Irregular phase combination will not often work, but they do with styrene–acrylonitrile.

Normal- and reversed-phase chromatography are like mirror images. It was a challenge to find out whether or not a given synthetic copolymer could be separated by both mechanisms. The first positive report appeared in 1987 when copolymers of styrene and ethyl methacrylate were measured by both modes of chromatography. All previous related work was by normal-phase separation. As expected, the elution order achieved by reversed-phase chromatography was the opposite of that in normal-phase chromatography. Since then, several polymer systems have been separated by normal-phase and reversed-phase chromatography with inversion of elution order, e.g. styrene–methyl methacrylate copolymers, styrene–methyl acrylate copolymers or methacrylate homopolymers graded in polarity of the ester group.

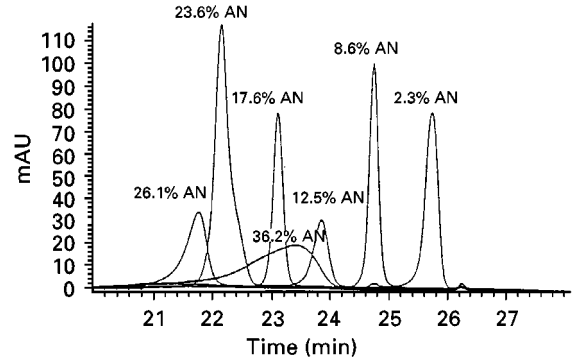

Figure 1 Merged plot of elution curves of seven styrene–acrylonitrile copolymers on a column (250 mm × 7.1 mm i.d.) packed with polystyrene gel. Gradient: methanol → tetrahydrofuran, 0–100% B in 25 min: UV signal detected at 254 nm. The acrylonitrile (AN) content of the samples is indicated on the curves; the amount of each injected was 30 μg. (Reproduced from Glöckner *et al.*, 1991, by courtesy of Vieweg-Verlag.)

High Performance Precipitation Liquid Chromatography of Styrene–Acrylonitrile Copolymers with Inversion of Elution Order

The separation of styrene–acrylonitrile copolymers with an elution order as in reversed-phase chromatography was achieved on a column packed with polystyrene gel. Eluent A was methanol (MeOH), a polar non-solvent for styrene–acrylonitrile samples; the less polar eluent, B, was either THF or dichloromethane. In both cases, the gradient rate was 0–100% B in 25 min. Copolymers with acrylonitrile content between 2.3% and 27.3% were retained longer the less acrylonitrile they contained (see **Figure 1**). Although the phase system and the elution order conformed to the rules of reversed-phase chromatography, the solubility mechanism prevailed.

The samples were prepared by copolymerization to only about 5% conversion, but they still consisted of macromolecules differing in composition. The chemical composition distributions of the samples are essentially responsible for the shape of the elution curves. The chemical composition distributions of samples with, say, 8.6% or 17.6% average acrylonitrile content are obviously narrower than that of a sample with 12.5% acrylonitrile.

The shape of the elution curve for 36.2% acrylonitrile in Figure 1 looks rather odd. In addition, the position of its maximum is not where it might be expected. According to its high acrylonitrile content, the sample is the most polar of the series investigated. It should therefore be eluted before the copolymer labelled 26.1% acrylonitrile, but is was eluted between the samples 17.6% and 12.5% acrylonitrile. This puzzling observation can be understood with the help of the solubility diagram for styrene–acrylonitrile in THF/MeOH, which is shown in **Figure 2**. The solubility boundary has a maximum at 20–25% acrylonitrile content, where samples require only about 45 vol% THF in MeOH for dissolution, whereas copolymers with more or less acrylonitrile need up to 12% more THF.

Along the left-hand branch of the solubility boundary (0–20% acrylonitrile), both polarity and solubility decrease with decreasing acrylonitrile content. The sequence of the five late-eluting peaks in Figure 1 is supported by polarity and solubility. Beyond the point of inflection, polarity increases but solubility decreases with increasing acrylonitrile content. A sample with 36.2% acrylonitrile requires about 50% THF for dissolution but should, according to polarity, already be released from the column in a mixture of 35% THF in MeOH. The measured peak position between the peaks for 17.6% and 12.5% acrylonitrile is determined by solubility. This is another indication of precipitation prevailing in the chromatography of styrene–acrylonitrile copolymers.

Along the left-hand branch of the solubility boundary, polarity supports the effect of solubility but beyond the turning point the two effects counteract each other. This is the reason why the elution curve for 36.2% acrylonitrile is much broader than the others. With normal-phase gradients, the 36.2% sample yielded an elution curve of the usual narrow shape, even in irregular phase systems (see **Figure 3**).

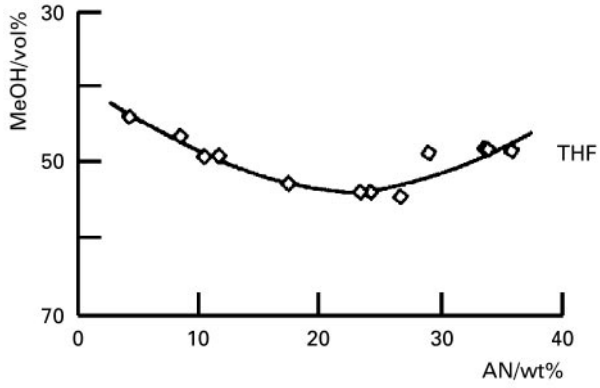

Figure 2 Solubility boundary for styrene-acrylonitrile copolymers in THF/MeOH as measured by turbidimetric titration at 20°C using THF as a sample solvent and methanol as the precipitating non-solvent. Phase separation (precipitation) occurs on crossing the curve from the upper part of the diagram (homogeneous solutions) to the lower part. (Reproduced from Glöckner *et al.*, 1991, by courtesy of Vieweg-Verlag.)

Sudden-Transition Gradient Chromatography of Synthetic Polymers

Interaction of Precipitation and Adsorption in Polymer Gradient Chromatography

Chromatographic retention and elution of synthetic polymers is generally governed by precipitation/dissolution and adsorption/desorption. The contribution of adsorption can be judged by comparing the solubility and elution characteristics of the sample system. If solubility prevails (and both temperature and concentration are suitable), both curves coincide. Noticeable adsorption shifts the elution characteristic above the solubility boundary, i.e. a higher concentration of solvent B is necessary for *eluting* a given sample than for *dissolving* it. The least adsorption was observed in reversed-phase systems with polystyrene samples. The predominance of an adsorption mechanism causes a retention behaviour different from (or even opposite to) that observed with a precipitation mechanism (see **Table 1**).

Baker and Williams reported that classical precipitation chromatography can be performed with 'a column of inert material … providing that the polymer gel does not flow through the column'. This type of flow can also occur in high performance precipitation chromatography in which case an optical detector may register the strong signal characteristic of a turbid liquid. Such a signal is affected by many parameters including time and is therefore poorly reproducible. Gel breakthrough can be avoided if there is some contribution of adsorption to retention. Separation according to composition with the least superimposition of molecular weight effects requires adsorption to dominate, i.e. elution by changing polarity of the eluent rather than by solvent strength. On the other hand, the unfavourable effect of sample size owing to strong adsorption can be compensated by increasing the contribution of precipitation to retention.

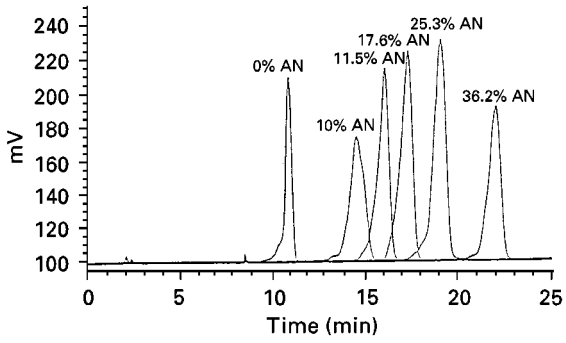

Figure 3 Merged plot of elution curves of six styrene–acrylonitrile copolymers on a reversed-phase column (250 mm × 4.3 mm i.d.) packed with C_{18} bonded phase; in irregular combination with a normal-phase gradient *n*-heptane → (THF + 20% methanol), 0–100% B in 25 min. Detection by signal from an evaporative light-scattering detector. The acrylonitrile (AN) content of the samples is indicated on the curves; the amount of each injected was 30 μg. (Reproduced from Glöckner *et al.*, 1991, by courtesy of Vieweg-Verlag.)

Independent Control of Adsorption and Precipitation

In common binary gradients, the solvent power and polarity of the mobile phase change simultaneously in the course of the run. An optimum can be sought by using a variety of different eluents A and B and their combinations. However, this is a cumbersome procedure requiring an adequate supply of chemicals and a prolonged time. In addition, it may not be successful because thermodynamic reasons restrict the number of possible solvents for a given polymer, and several of these may be further ruled out by physical, physiological, or financial reasons. More promising and efficient is the use of ternary systems consisting of two non-solvents (A and B) and one solvent (C) for the polymers under investigation. A and B must be opposite in polarity, i.e. if A is a polar non-solvent, B is a nonpolar one. The polarity of solvent C is in between those of A and B. Solvent C must be miscible with A and B and must have sufficient strength to dissolve samples in the whole range of molecular weight and composition under investigation.

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Table 1 Features of polymer gradient chromatography with predominance of either precipitation/dissolution or adsorption/desorption

Dominating mechanism	*Elution characteristic*	*Irregular phase combinations*	*Increasing temperature*	*Increasing sample size (overload)*	*Increasing molecular weight*[a]
Percipitation	Coincident with the solubility boundary	Separating like standard ones	Decreases retention	Increases retention	Increases, retention, $C_2 = 2\text{–}4 \times 10^3$
Adsorption	Above the solubility boundary	Ineffective in separation	Increases the retention of polymers	Decreases retention	Increases retention slightly, $C_2 = 2\text{–}5 \times 10^2$

[a]For C_2 factors see the equation in the text. Values of C_2 are compiled in Glöckner G (1991) *Gradient HPLC of Copolymers and Chromatographic Cross-Fractionation*, p. 107. New York: Springer-Verlag.

Since with gradients of this kind the chromatographically significant process is the result of interactions between non-solvents there is, owing to the large variety of the latter, more freedom in adjusting optimum conditions than with binary non-solvent/solvent gradients.

The samples to be investigated are dissolved in solvent C and injected into a starting eluent (e.g. A), whose polarity and precipitating power ensure proper retention at the top of the column. Solvent C is then added to the eluent at a concentration that in itself does not suffice for elution. In order to achieve short chromatograms, the concentration of C is changed as rapidly as the apparatus allows. No unfavourable side effects of the shock caused by the sudden transition from injection to elution conditions have ever been observed. The disturbance is visible with the help of optical detectors. With cyanopropyl or C_{18} packings, it is swept through by the approximately three-fold volume of mobile phase in the column. The elution of the sample is then triggered by a gradient A → B at a constant level of solvent C.

The first results of gradient elution with sudden transition of solvent concentration were achieved in the normal-phase mode of chromatography. The column packing was polar (CN-modified silica), A was iso-octane (with addition of 2% MeOH to the starting eluent), B was MeOH and C was THF. The gradient A → B was performed at 5% min^{-1} and applied to copolymers of styrene and ethyl methacrylate (EMA), methyl methacrylate (MMA), or 2-methoxyethyl methacrylate.

Figure 4 is the merged plot of UV signals measured on the elution of a mixture of five styrene–EMA copolymers through a gradient iso-octane → MeOH after sudden transition to 20, 25, 30 or 35% THF solvent. Both iso-octane and MeOH are non-solvents for styrene–EMA. The addition of 35% THF yielded too high a solubility: the sample with 4.7% EMA was swept through the column by the sample solvent. A proportion of 20 or 25% THF did not suffice for baseline separation. The best result was obtained after addition of 30% THF.

The advantage of this technique in comparison with binary gradient elution is obvious (see **Figure 5**). The chromatogram in Figure 5 was obtained in the same laboratory as those of Figure 4 with the same instrument and identical solvents. The baseline shift in Figure 5 is due to the UV absorption of THF which, at 259 nm, is slightly higher than that of iso-octane. This causes the baseline rise with

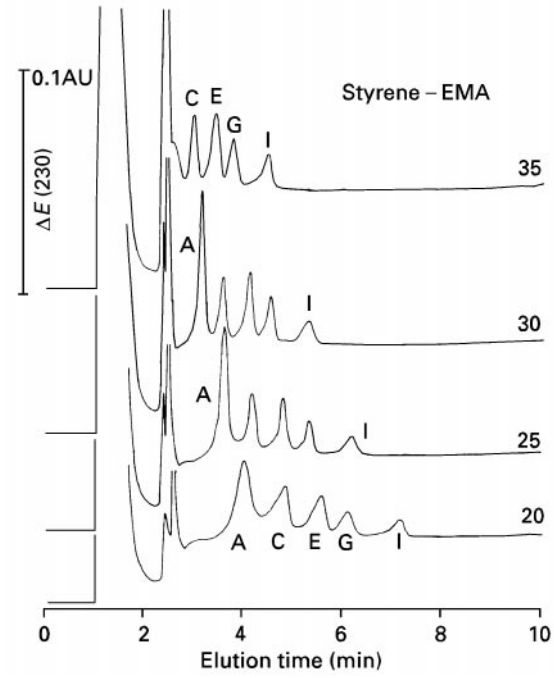

Figure 4 Separation of the mixture of five styrene–EMA copolymers at 50°C on a column (60 mm × 4 mm i.d.) packed with cyanopropyl bonded phase. Gradient: *iso*-octane → methanol (5% min^{-1}) after increase of THF concentration from zero to the percentage indicated at the curves; flow rate 0.5 mL min^{-1}. Sample: 1.8 μg copolymer A (4.7% EMA) + 1.2 μg C (32.2% EMA) + 2.0 μg E (54.6% EMA) + 1.2 μg G (68.0% EMA) + 2.0 μg I (92.5% EMA); UV signal detected at 230 nm. (Reproduced from Glöckner, 1991, by courtesy of Springer-Verlag.)

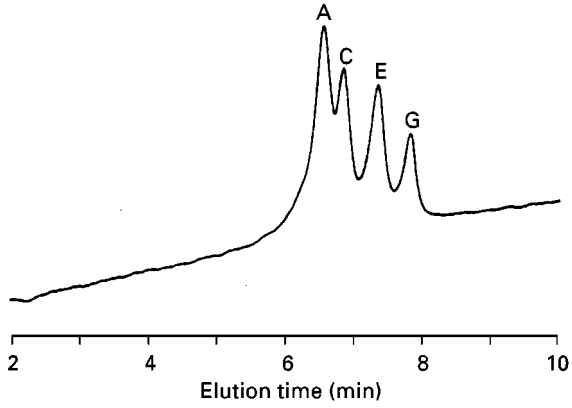

Figure 5 Separation of the mixture of four styrene–EMA copolymers at 50°C on a column (60 mm × 4 mm i.d.) packed with silica. Gradient: iso-octane → methanol (5% min^{-1}); flow rate 0.5 mL min^{-1}. Samples A to G as in Figure 4, 2.5 μg each; UV signal detected at 259 nm. (Reproduced from Glöckner, 1987a, by courtesy of Elsevier Science Publishers.)

increasing THF content of the eluent. The effect would be still more dramatic at a shorter wavelength, e.g. at 230 nm. Figure 4 presents horizontal baselines although the chromatograms were monitored at 230 nm. This is due to the constant concentration of THF throughout the elution, which disturbs the traces much less than a changing amount of THF does. The higher the THF addition, the higher the level of the baseline at the end of the chromatogram in comparison to the starting position in Figure 4.

The poor separation in Figure 5 is explained by the comparatively low molecular weight of these samples ($50–80 \times 10^3$) and the superimposition of separation by molecular weight and by composition. The peaks are indeed quite well separated when SEC fractions of the copolymer mixture are injected. Figure 4 indicates that the molecular weight effect in the investigation of the raw copolymers can be suppressed by the sudden-transition technique. **Table 2**

Table 2 Characteristics of polymer separation with separate control of solubility and adsorption[a]

Factor	*Details*
Sample	20–100 μg polymer per injection, dissolved in about 50 μL solvent
Solvent	C, capable of dissolving samples of the system under investigation in the whole range of composition and molecular weight, used also for sample solutions (recommended: tetrahydrofuran, dichloromethane)
Non-solvents	A and B, opposite in polarity, both miscible with solvent C, e.g. A, acetonitrile, methanol; B, *n*-heptane. In general, the variety of non-solvents for a given polymer system is much broader than the list of suitable solvents
Interactions of eluents and detector	Eluents must not impede the monitoring of the eluting sample components, they must be transparent if optical detection is employed. This demand is more stringent for the gradient components A and B than for solvent C, whose concentration is not changed during the elution of sample components. For instance, separations at constant concentration of THF can be monitored at 230 nm or at constant DCM concentration with an evaporative light scattering detector without disturbance
Reversed-phase separation	Non-polar column, e.g. reversed-phase C_{18} bonded phase, injection into polar non-solvent A, gradient A → B after adjusting the solvent concentration to a suitable constant value
Normal-phase separation	Polar column, e.g. cyanopropyl bonded phase, injection into non-polar non-solvent B, gradient B → A after adjusting the solvent concentration to a suitable constant value
Reversed-phase and normal-phase separations	Can be performed with a common set of three eluents
Automated search for optimum separation method	Possible with programmable apparatus equipped with three storage bottles and a device for column switching
Balance between solubility and adsorption	Can be adjusted by the solvent concentration, which remains constant during the elution
Length of chromatograms	Can be optimized by sudden transition[a] of solvent concentration from zero to the selected level

[a]Information on how to perform sudden-transition gradients is available in Glöckner G, Wolf D and Engelhardt H (1994) *Chromatographia* 39: 557–563.

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summarizes the characteristics of sudden-transition gradient elution.

Chromatography in Normal-Phase and Reversed-Phase Modes Using a Solvent and Two Non-Solvents

Independent control of adsorption and solubility enables normal-phase *and* reversed-phase separations to be performed with a common set of three liquids. This was first demonstrated with styrene–MMA copolymers in the system A (acetonitrile), B (*n*-heptane) and C (dichloromethane, DCM) on either CN or C_{18} bonded phases.

Figure 6 shows chromatograms measured under reversed-phase and normal-phase conditions. Both modes yielded good separations. The elution order is inverted in the reversed-phase mode, as expected. The elution of styrene–MMA copolymers by the strong precipitant heptane (Figure 6A) is rather surprising.

Figure 7 shows the composition triangle of the eluent system used in Figure 6 with dichloromethane at the top, the polar non-solvent acetonitrile at the bottom left and the non-polar precipitant heptane at the bottom right. Acetonitrile and heptane have a miscibility gap that diminishes as dichloromethane is added. Eluent mixtures containing 25% or more dichloromethane are homogeneous. The elution characteristics of the styrene–MMA copolymers investigated in reversed-phase mode with 25–50% DCM or in normal-phase mode with 25–40% dichloromethane are indicated. The characteristics of reversed-phase elutions form a group in the left-hand area of the triangle. The proportion of acetonitrile present means that eluent systems in this region have a higher polarity than those in the right-hand region. Reversed-phase chromatography starts with retention in a strongly polar medium. Sample components are released when the polarity of the eluent is no longer sufficient for retention. Thus, the characteristics of reversed-phase elution are to be expected on the polar side of the composition diagram. On the other hand, normal-phase elution characteristics are located in the right-hand part of the triangle. This can be understood by complementary reasoning because normal-phase chromatography starts with retention in a nonpolar medium.

The characteristics in Figure 7 are due to samples containing methyl methacrylate in the proportions (from left to right) 83.7%, 62.2%, 48.1%, 34.1%, 14.1% or 0% (polystyrene homopolymer). This sequence holds true with reversed-phase as well as with normal-phase elutions. In both modes, the copolymer with the highest content in polar methyl methacrylate units yields characteristics nearer to the polar (left) side of the diagram than the other samples. As expected, the least polar sample (polystyrene) marks the right border of the elution area in each mode.

All polymers considered here are soluble in the region beneath the solvent apex. The addition of

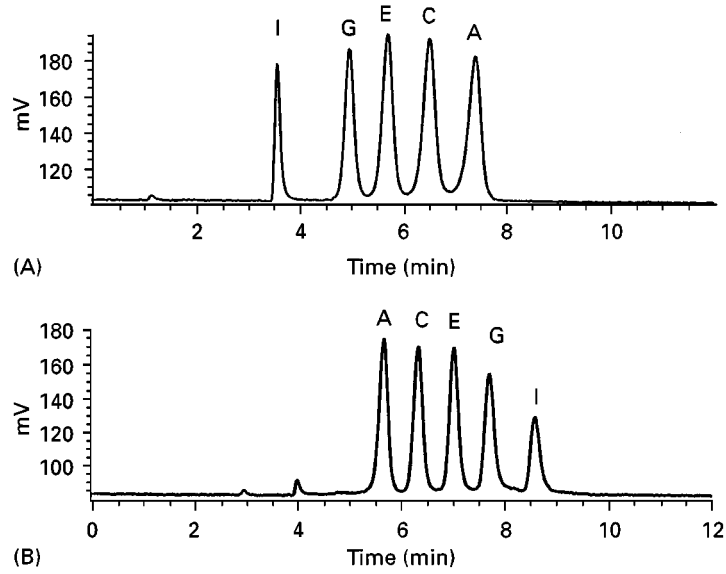

Figure 6 Separation of the mixture of five styrene–MMA copolymers at 35°C and flow rate 1 mL min^{-1} by gradient elution in reversed-phase (A) or normal-phase mode (B) after a sudden increase of dichloromethane concentration from zero to 30%, monitored by an evaporative light-scattering detector. Sample in each mode: 6.76 μg copolymer A (14.1% MMA) + 5.54 μg C (34.1% MMA) + 5.28 μg E (48.1% MMA) + 5.48 μg G (62.2% MMA) + 5.02 μg I (83.7% MMA), dissolved in 10 μL DCM. (A) Column (250 mm × 4.1 mm i.d.) packed with reversed-phase C_{18} bonded phase. Gradient: acetonitrile → *n*-heptane (4.99% min^{-1}). (B) Column (250 mm × 4.1 mm i.d.) packed with cyanopropyl bonded phase. Gradient: *n*-heptane → acetonitrile (4.99% min^{-1}). (Reproduced from Glöckner *et al.*, 1994, by courtesy of Vieweg-Verlag.)

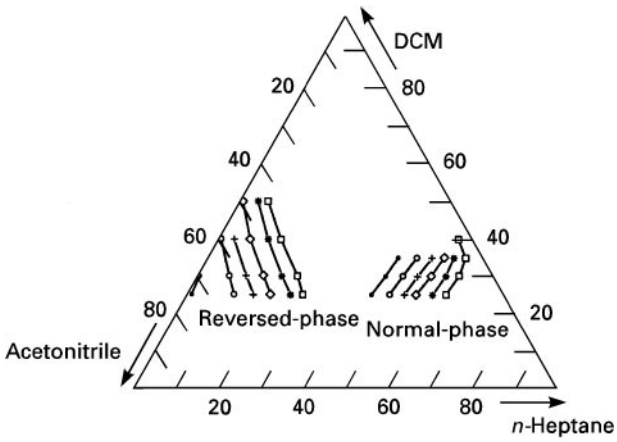

Figure 7 Composition triangle for acetonitrile/*n*-heptane/dichloromethane with elution characteristics of styrene–MMA copolymers in normal-phase and reversed-phase sudden-transition gradients. Samples: ●, 83.7% MMA; ○, 62.2% MMA; +, 48,1% MMA, ◇, 34.1% MMA; ∗, 14.1% MMA; □, polystyrene. (Reproduced from Glöckner, 1996, by courtesy of Gordon & Breach.)

small quantities of heptane or acetonitrile to dichloromethane will impair solubility but will not immediately cause precipitation. The polymers are still soluble in mixtures of dichloromethane with about 40% acetonitrile or 40% heptane. Thus, the upper sections of the solubility boundary follow the left and right sides of the eluent triangle. With increasing concentration of nonsolvent, a precipitation threshold is reached on each side. From these points, both branches of the solubility boundary bend towards each other. These sections may be determined experimentally by turbidimetric titration. For example, the elution characteristics of the copolymer containing 48.1% MMA run almost parallel to the corresponding sections of the solubility boundary. In both reversed-phase and normal-phase modes, the elution characteristics are shifted from the solubility boundary towards the centre of the solubility window. This shift indicates the contribution of adsorption to retention, which is well known in gradient HPLC of styrene–methyl methacrylate copolymers. Finally, both branches of the boundary will merge inside the triangle (above the miscibility gap). For details, see Glöckner G (1996).

Solubility windows of similar shape can be expected with many polymers in mixtures of a solvent with two non-solvents differing in polarity. Hence, HPLC separation generally should be possible in normal-phase as well as in reversed-phase mode with a suitable ternary eluent system. These separations should be achievable near the respective side of the solubility boundary. Thus, the use of ternary gradients consisting of a solvent and two non-solvents and control of solubility by a sudden increase of solvent concentration to a constant level will not only offer the opportunity to improve separations with small additional effort, but will also contribute to a better understanding of the mechanisms of polymer chromatography.

See also: **II/Chromatography: Liquid:** Mechanisms: Normal Phase; Mechanisms: Reversed Phases; Mechanisms: Size Exclusion Chromatography. **III/Polyethers: Liquid Chromatography. Synthetic Polymers:** Liquid Chromatography.

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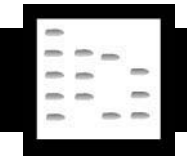
HERBICIDES



Gas Chromatography

J. L. Tadeo and C. Sanchez-Brunete, Department of Sustainable Use of Natural Resources, INIA, Madrid, Spain

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Herbicide Formulations

Weeds have been controlled by humans since the beginning of agriculture by means of mechanical tools or by hand. It was early in the 20th century that some inorganic compounds were first used with this aim. The discovery of the herbicidal properties of 2,4-D (2,4-dichlorophenoxyacetic acid) in 1945 can be considered the initiation of use of organic herbicides in agriculture. Since then, more than 130 different active compounds have been synthesized for their application as herbicides. These compounds can be grouped, according to their chemical structures, into different herbicide classes (**Table 1**).

Compounds belonging to the principal herbicide groups will be considered in this study. These compounds control weeds in a variety of ways, showing different modes of action, selectivity and application characteristics. Soil-applied herbicides are absorbed by roots or emerging shoots and foliage-applied herbicides are absorbed into the leaves, where they may be translocated to other parts of the plant.

The active ingredient of a herbicide is a compound, usually obtained by synthesis, which is formulated by a manufacturer in soil particles or liquid concentrates. These commercial formulations of herbicides are diluted with water before application in agriculture at the recommended doses. Herbicide formulations generally contain other materials to improve the efficiency of application.

Analysis of herbicide formulations was initially carried out by wet chemical procedures, such as determination of total chlorine, nitrogen or phosphorus, or by spectrometric procedures like ultraviolet absorption. The development of gas chromatography (GC) allowed the analysis of these compounds in commercial formulations with high selectivity and sensitivity. The analytical procedure is commonly based on the dissolution of a known amount of the formulation in an organic solvent, which often contains an internal standard to improve the precision and accuracy of the determination. An aliquot of this solution is analysed by GC. Packed columns were used initially, but have now been replaced by capillary columns of low or medium polarity and flame ionization is the detection technique more widely used. When herbicides are not volatile or thermally stable, high performance liquid chromatography (HPLC) is the preferred technique for their determination in commercial formulations. **Figure 1** shows the gas chromatographic separation of a mixture of phenoxy esters.

Herbicide Residue Analysis

Residues of herbicides will persist in the plant or in the soil for a variable time, depending on their physicochemical properties and on the environmental conditions. Analysis of herbicide residues in these matrices is important, not only from the point of view of the efficacy of application, but also to know the distribution and persistence of these compounds in food and in the environment. Therefore, herbicides of a wide range of polarities have to be determined in complex environmental matrices at very low levels.

Initially, herbicide residues were analysed by colorimetric methods. These procedures were generally based on acidic or basic hydrolysis followed by formation of derivatives. These methods are time-consuming and do not usually distinguish between the parent herbicide and metabolites.

Since the development of GC, this technique has been widely used in the analysis of these compounds. **Table 2** summarizes the preparation of different types of samples for residue determination. These samples are generally analysed by a procedure with the following main steps: sample extraction, clean-up of extracts, then GC determination and identification. Some compounds are not volatile or thermally stable